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Studies on *Pasteurella multocida:* Indirect Haemagglutination Test for the Identification of Serological Types

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Abstract: Haemorrhagic septicaemia (HS) is an acute, infectious disease of cattle and buffaloes, caused by a bacterium *Pasteurella multocida.* All of the isolates of *P. multocida* gave consistent results for nitrate reduction test, methyl red test, catalase test, indole production test and gelatin liquifiction test. Variable results were obtained for H_2S production test. Sugar fermentation tests were uniform for sucrose, glucose, mannose, fructose and salicin but were variable for lactose and maltose. The results of IHA showed that all of the thirteen isolates agglutinated with the anti-serum raised against Roberts Type I in rabbits. It has been concluded that prevalent serotype of *P. multocida* causing heamorrhagic septicemia in cattle and buffalo in Faisalabad, Pakistan is Roberts type I, which-is equivalent to Carter's type B.

Key words: Haemorrhagiesepticemia, Indirect haemagglutination test, *Pasteurella multocida*

Introduction

Haemorrhagic septicaemia (HS) commonly effects cattle and buffalo in Southern Europe, North, Central and East Africa, the Southern and South East Asia, including Pakistan. Morbidity due to this disease is more than 45% and mortality is 92% in diseased animals. In Pakistan HS is causing a loss of Rs. 1.8 billion rupees per annum. The prevalence of disease varies from region to region. In tropical countries, the greatest incidence is in the rainy season, although isolated cases may occur at any time 'during the year. It is suggested that some extraneous factor is necessary to precipitate the outbreak of disease. The causative bacterium is known as *Pasteurella multocida.* It has been reported to occur normally in the respiratory tract of healthy animals. Disease appears when the resistance of the animal is lowered. The strains of *P. multocida* are classified on the basis of capsular and somatic antigens. An indirect haemagglutination test (IHA) identifies five capsular groups A, B, D, E and F (Carter, 1955; Rimler and Rhoades, 1987), a gel diffusion precipitin test identifies 16 somatic types 1 to 16 (Heddleston *et al*., 1972; Brogden *et al*., 1978) and less commonly used agglutination test identifies 12 somatic types 1 to 12 (Namioka and Murata, 1961; Namioka, 1970). Capsular serogroups B or E and somatic serotype 2 (Namioka serotype 6) *Pasteurella multocida* are recognized as the cause of a specific disease, Haemorrhagic septicaemia. Serogroup B is widely distributed but serogroup E has been reported only in Africa (Carter and De-Alwis, 1989). The Carter's types B is equivalent to Heddleston's type 2, Robert's type I and Namioka and Murata's type 6: B. The present study was carried out to know the prevalent strain of *P. multocida* in Faisalabad by using IHA test. It will be helpful for the control and prevention of the disease because through this study; any change could be identified and be necessary to known any mutation in the strain for successful vaccination programmes.

Materials and Methods

Sample collection: The blood samples were derived from the jugular veins of cattle and buffaloes aseptically showing typical signs of Haemorrhagic septicaemia. Culturing the blood sample in broth media and then on agar plates did the isolation. The isolated colonies on the agar plates were selected and slides were prepared and stained by Gram's staining for morphology and India ink staining for capsule identification. Sub cultured purified colonies having characteristic morphology of *P. multocida* but repeated sub culturing was avoided.

Studying its cultural characteristics on various media including nutrient agar, blood agar, MacConkey agar and citrate agar did the confirmation of the organism. Growth of the organism, size of colony, pigmentation and their ability to produce any change in the medium like haemolysis on blood agar was examined. Urease activity on urea agar, fermentation of sugar (Lactose, Maltose, Salicin, Mannose, Sucrose, Fructose, Glucose) in peptone water, gelatin liquifiction test, H_2S production test, catalase test, indole production test, methyl red test and nitrate reduction test were also observed.

The pathogenicity of *P. multocida* was observed by inoculating 6-8 hour growth of *P. multocida* into rabbits.

Standard methods of media preparation and biochemical tests described by Buxton and Fraser 1977, Wijewardana 1992 and Cruickshank 1988 were followed.

Serotyping: The serotyping of biochemically confirmed isolates was done by indirect haemagglutination test.

Preparation of hyperimmune rabbits antiserum: A 6-8 hour broth culture of the- reference strain was seeded onto casein sucrose yeast extract agar and incubated at 37°C overnight. Growth on each plate was checked for its purity by the rapid slide agglutination test. The growth was harvested by washing the plates using 2-3 ml per plate of a 0.3 percent formalinized buffered saline. The turbidity was adjusted by spectrophotometer at 640 nm corresponding to approximately 10^9 organism/ml. Two mature rabbits were inoculated by the intravenous route (I/V) with the suspension of already prepared antigen. The inoculation schedule consisted of 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 ml volumes respectively at 4-day intervals. Seven days after the last inoculation 0.5 ml of a live 6-hour broth culture of the reference strain was injected intravenously. Rabbits were bled from the ear vein 10 days after this injection. The serum was then separated and stored at -20°C.

Preparation of antigen for IHA: To separate the capsular antigen, the bacterial suspension was given heat treatment at 60°C for 30 minutes. After heat treatment, suspension was centrifuged at 2000 rpm for 30 minutes and supernatant was collected. The optimal dilution of the antigen for the sensitization of human '0' erythrocytes was determined by carrying out an antigen titration against hyper immune rabbit antiserum. The antigen dilution used

in the IHA test was fixed as 2 haemagglutination units. Indirect haemagglutination test was performed by the procedure described by Carter (1955).

Results and Discussion

On account of multi host nature, organism is heterogeneous in its characteristics with considerable difference in host predilection, pathogenicity, biochemical activities, colonial morphology and antigenic structure. Therefore, the effective control of HS in cattle and buffalo by the use of type specific vaccine requires a comprehensive knowledge about the various serotypes of *P. multocida* prevailing in particular area.

Out of 28 blood samples *P. multocida* could be isolated from 13 samples. *P. multocida* from blood samples was difficult to grow on solid media directly as compared to broth media. Colonies on CSY agar were round sticky and were of mucoid consistency, slightly raised in the center, on blood agar non hemolytic, on MacConkey agar and citrate agar no growth could be observed. Microscopic examination revealed that the organisms were bipolar coccobacilli. With repeated sub culturing organism tend to diminish in size and became some what rounded and sometimes even lost its bipolar character. All of these observations were in agreement with those studied by Bain *et al.* (1982) and Wilson *et al.* (1984).

All the isolates fermented glucose. This has also been reported by Aslam *et al.* (1988). In case of sucrose and mannose all the isolates were able to ferment while salicin was not fermented by any of the isolate. These results were similar to Mohan *et al.* (1994). Fermentation of maltose and lactose was variable in our study, similar to the studies of Kozarev and Mamadudian (1988). All the isolates were positive for indole production test and also for catalase test but were negative for methyl red test, urease test, and gelatin liquefaction test. The similar results were obtained by Aslam *et al.* (1988).

Serotyping was done on the basis of capsular antigens employing ever reliable and simple method of indirect Hemagglutination test. All of the isolates showed agglutination reaction with the serum raised against Robert's type I which showed that all of the isolates collected from Faisalabad district belonged to Robert's type I which was thought to be equivalent to Carter's type B. These results are also in agreement with those obtained by Ahmad and Anjum (1972) and Ajmal *et al.* (1985) who conducted investigation on serotypes prevalent in Pakistan. Aslam *et al.* (1988) proved that only Robert's type I is prevalent in Pakistan.

This study confirmed the previous findings and proved that there is only one serotype of *P. multocida* responsible for Haemorrhagic septicaemia in and around Faisalabad. Screening of prevalent bacterial strains in any particular area would be regular procedure to detect the bacterial mutation, if any in the area, because it is necessary for successful vaccination programmes for control and eradication of disease from livestock.

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